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14. ABSTRACT: Sleep disruptions are a common clinical feature observed in children with autism spectrum disorders (ASD). These include irregular sleep-wake patterns, delayed sleep latencies, and problems with sleep maintenance. The etiology of these sleep disturbances is unknown and remains relatively unexplored in any animal model of ASD. Prenatal valproic acid (VPA) exposure is a proposed model of ASD. Pups exposed to VPA *in utero* show similar characteristics to children with ASD, including abnormalities in brain morphology and sex-specific behavioral deficits. With this model, we examined the sleep architecture of prenatally, VPA- treated juvenile rats (PN31-34). We used a telemetry system to record electroencephalogram and electromyogram activity of each animal. Two 12-hour light phases (PN 31 and PN 34) were manually scored for each vigilance state and the data were averaged for each animal. VPA-treated animals showed a change in sleep patterning, with more consolidated bouts of wake (60.1% increase in average bout length; $t_{(11)}=5.783$, $p=0.0001$) and non-REM sleep (21.8% increase; $t_{(11)}=4.066$, $p=0.0019$) as well as fewer transitions into wake and non-REM sleep. There was a significant decrease in the number of transitions from wake to non-REM sleep (36%; $t_{(11)}=8.657$, $p<0.0001$), non-REM sleep to wake (30.7%; $t_{(11)}=6.974$, $p<0.0001$), and REM sleep to wake (52.8%; $t_{(11)}=5.712$, $p=0.0001$). Total sleep time and delta power, however, were similar in VPA-treated and controls animals. While these disruptions did not alter the total amount of acquired sleep in the animal's sleep phase, the alterations in patterning may disrupt sleep-dependent functions such as metabolic homeostasis, maintenance of the stress axis and synaptic plasticity. ***To our knowledge this is the first report of an ASD animal model mimicking clinical sleep disruption.*** By better understanding the consequences of disrupted sleep patterning, potential sleep therapies may be used to help alleviate some of the symptomology associated with ASD.

15. SUBJECT TERMS
Sleep architecture, Arousal, Non-REM sleep, Slow wave sleep, Valproic acid, Animal model of Autism Spectrum Disorder,

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Introduction

Recent clinical studies report that the prevalence of sleep problems in children with Autism Spectrum (ASD) Disorders is 44% to 83% of diagnosed cases [1-4]. These sleep dysfunctions typically manifest as difficulties initiating and maintaining sleep, sleep fragmentation and insomnia. Quality sleep is imperative for the maintenance of good health. Non-ASD children and adolescents suffering from sleep disordered are not only fatigued but have impaired memory and learning, increased irritability, hyperactivity, inattention and aggressiveness leading to increased stress and anxiety and a decreased quality of daily life [5]. Children diagnosed with a ASD share similar symptoms. Thus, it is plausible to suspect that sleep disturbances in ASD may contribute to its pathology. In fact, because sustained states of hyperarousal and sleep disruption can lead to increased stress and anxiety, it is likely that disturbed sleep contributes to the development and maintenance of the aforementioned behaviors. ***A question that remains unanswered is whether the neurocircuitry underlying the sleep pathways is developmentally changed in ASD or whether disruptions in sleep are a consequence of changes in daily anxiety levels.*** Distinguishing between the two possibilities in a clinical setting would be challenging. Amazingly, rodent's neurocircuitry and neurochemistry of sleep share similarities suggesting that rats would be a good model system for basic investigations. ***Surprisingly, to date, the current animal models of ASD have not been utilized to investigate the underlying causes of sleep disturbances.*** In the current concept award, we hypothesized that alterations in cytokine function are involved in disruption of sleep states in ASD. To test this we prenatally exposed rat fetuses to valproic acid and tested (i) whether sleep patterns were disrupted, (ii) whether cytokine levels were changed and if so (iii) whether blocking the cytokines would correct the sleep and finally (iv) whether other neurochemical parameters of sleep were affected. We have found that indeed sleep patterns in the VPA model are changed compared to control animals. Additionally, in the basal forebrain, a brain area involved in sleep-wake, we have found an increase in the proinflammatory cytokine, TNF-alpha. However, we were not able to correct sleep dysregulation by blocking TNF-alpha signaling. These results are described in detail below and to our knowledge are the first demonstrations of sleep disturbances in an animal model of ASD.

Body

As described in the original application, it is necessary to breed the animals in house because the VPA treatment is given on embryonic day 12.5. Unfortunately, due to unknown circumstances our animal colony developed breeding problems. Approximately, 4 months from the start of the project successful delivery became sporadic and eventually stopped altogether. The problem was resolved. We requested a new room in our animal facility that was more secluded. This temporary interruption in available animals slowed our progress but we were able to test the predictions as outline below.

Task 1: Examine changes in sleep architecture in male and female rats prenatally exposed to valproic acid (VPA).

Design: In this task, pregnant dams received a single i.p. injection of 600mg/kg of sodium valproate on GD12.5, control females were injected with physiological saline. The dams were allowed to deliver and the pups were raised to postnatal day 35. At this time point, animals were surgically implanted with DSI telemeters and EEG recording electrodes were placed on the skull surface over the parietal cortex. After a recovery period (typically 5 days) the animals were reintroduced to littermate (not surgically implanted) in their homecage and the EEG acquisition was started. We collected continuous EEG data for 4 day. The EEG tracings are manually scored for sleep states. Once scored the automated software analysis parameters of sleep architecture. The results are presented in the next section.

Results:

VPA treated animals appear to have consolidated bouts of Wakefulness during their subjective night or sleep phase (i.e. Light Phase). Visual inspection of the software generated hypnograms reveal that VPA animals have longer bouts (i.e. consolidated) of wake compared to the saline treated controls (Figure 1).

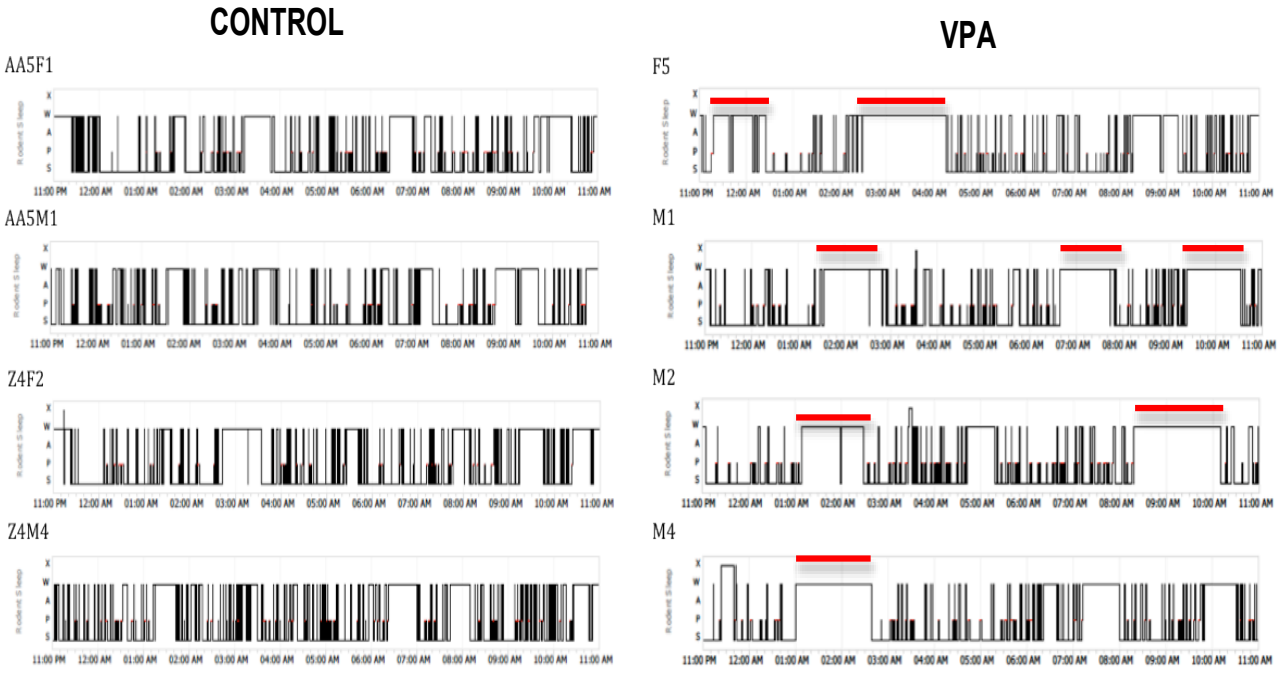

Figure 1: Hypnogram of sleep-wake behavior in the Light (sleep) phase in 4 representative animals from the Control and VPA treated groups. The x-axis represents the scored sleep states (W: Wake; P: REM as referred to as paradoxical sleep; and S: Slow wave or NREM sleep). The Red line above the Wake periods represents bouts of consolidated Wake that are statistically longer in the VPA than Control animals.

Total time spent in WAKE, Non Rapid Eye Movement (NREM) sleep, or REM sleep during the light phase was not significantly different between VPA and control treated animals. The total number of minutes spent in each state was summed across the 12 hours of light (Sleep Phase). Surprisingly, we did not detect a significant difference between VPA (n=12) or Control animals (n=12) (Figure 2; unpaired t-test)

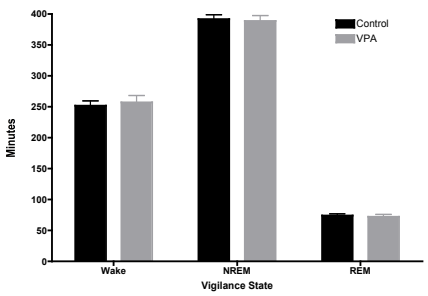

Figure 2. Total time (in minutes) spent in each vigilance state during the light phase No significant differences between controls and VPA treated animals were detected.

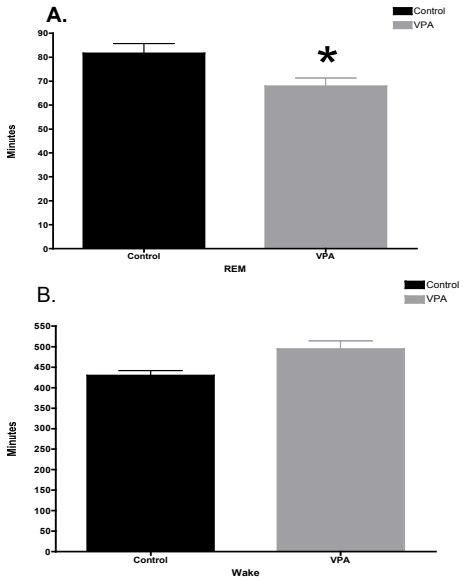

Figure 3. Total time spent in REM (A) and Wake (B) over the 12 hour dark phase. Significant difference in REM sleep were observed; VPA treated animals spent less time in REM ($t_{(9)}$=2.636; p=0.0271). These same animals had increases in wakefulness.

Total time spent in REM sleep during the Dark phase (active) was significantly different between VPA and control treated animals. The total number of minutes spent in wake, NREM and REM sleep was summed across the 12 hours of dark (active Phase). VPA treated animals spent less time in REM than control (Figure 3A; unpaired t-test $t_{(22)}$=2.636; p=0.0271). Although not significantly different, the VPA animals exhibited increased wake (Figure 3B). This observation suggest that the during the animals active phase, the VPA animals may be hyper-aroused. We analyzed the EEG Power Spectra (activity data) from these recordings to test this prediction.

VPA-exposure results in changes in the power spectra

Power spectral analysis was utilized to study the cortical EEG of the juvenile rats exposed to VPA or SAL in utero during waking, NREM, and REM sleep. Overall, VPA exposure reduced mid-range frequencies while increasing higher range frequencies primarily in wake and REM sleep; the two states characterized by EEG desynchrony (Figure 4 and Table 1). Across all states, VPA exposure decreased theta band densities (4-8hz) in both the light phase and the dark phase (Fig. 4A-C). Most notable was the approximate 20% decrease in theta band density during REM sleep regardless of phase (Fig. 4C). Alpha band densities (8-12hz), were also significantly decreased during wake in the

Table 1: Mid-range power spectral analysis. Bolded means ± SEM represent significant differences between Saline and VPA exposure groups for that phase.

WAKE				
	Light Phase		Dark Phase	
Power Band (Hz)	Saline	VPA	Saline	VPA
Alpha (8-12)	**6.3 ± 0.1**	**5.7 ± 0.1**	**6.9 ± 0.0**	**6.0 ± 0.1**
Sigma (12-16)	**2.6 ± 0.04**	**2.8 ± 0.05**	**2.7 ± 0.05**	**2.9 ± 0.05**
Beta (16-24)	3.5 ± 0.2	3.9 ± 0.2	**3.7 ± 0.1**	**4.1 ± 0.1**
NREM				
	Light Phase		Dark Phase	
Power Band (Hz)	Saline	VPA	Saline	VPA
Alpha (8-12)	5.9 ± 0.3	6.1 ± 0.3	6.1 ± 0.4	6.3 ± 0.3
Sigma (12-16)	2.4 ± 0.2	3.3 ± 0.3	**2.5 ± 0.2**	**3.4 ± 0.3**
Beta (16-24)	2.1 ± 0.2	2.6 ± 0.3	2.4 ± 0.3	2.8 ± 0.2
REM				
	Light Phase		Dark Phase	
Power Band (Hz)	Saline	VPA	Saline	VPA
Alpha (8-12)	7.8 ± 0.6	7.4 ± 0.3	8.5 ± 0.6	7.6 ± 0.3
Sigma (12-16)	**3.2 ± 0.3**	**4.0 ± 0.2**	3.2 ± 0.2	3.9 ± 0.3
Beta (16-24)	**4.0 ± 0.4**	**5.3 ± 0.3**	**4.0 ± 0.2**	**5.4 ± 0.2**

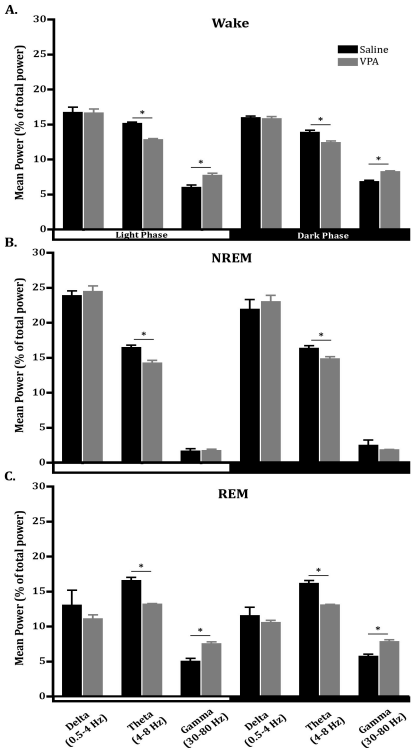

Figure 4. Power analysis revealed VPA exposure increased EEG frequencies associated with cognitive arousal and cortical activity (see Gamma) in both the light and dark phases

light (9.5%) and dark (13.%) phases in VPA exposed rats compared to SAL exposed controls (Table 2).

Sleep architecture was markedly different between the VPA and control animals.

Although the VPA treated animals acquired similar amounts of total wake and sleep, it was clear from the hypnograms (Figure 1) that the sleep patterns were severely disrupted. To quantify these apparent differences we analyzed the mean bout length, bout number and transitions into the various vigilance states in the light and dark phases. VPA exposure significantly increased the mean duration of a wake bout in the light and dark phases by 50% and 60%, respectively compared to the SAL-exposed animals ($t(9)=6.966$, $p<0.0001$, light phase and $t(9)=2.552$, $p=0.03$, dark phase; Figure 5A). The increase in bout duration was associated with a significant decrease in the number of wake bouts in the light phase only [$t(9)=6.532$, $p=0.0001$, Fig. 5D].

For sleep, only NREM sleep demonstrated significant changes in architecture. Curiously, in the light phase, VPA exposure significantly increased the average duration of a NREM sleep bout [$t(9)=3.517$, $p=0.007$; Fig. 5B] while the number of NREM bouts was significantly reduced compared to SAL-exposed animals ($t(9)=6.646$, $p<0.0001$, Fig. 5E). For REM sleep, there was no significant differences in the mean bout duration or bout number in either phase; however in the dark phase there was trend in the bout duration (Fig 5C, D)

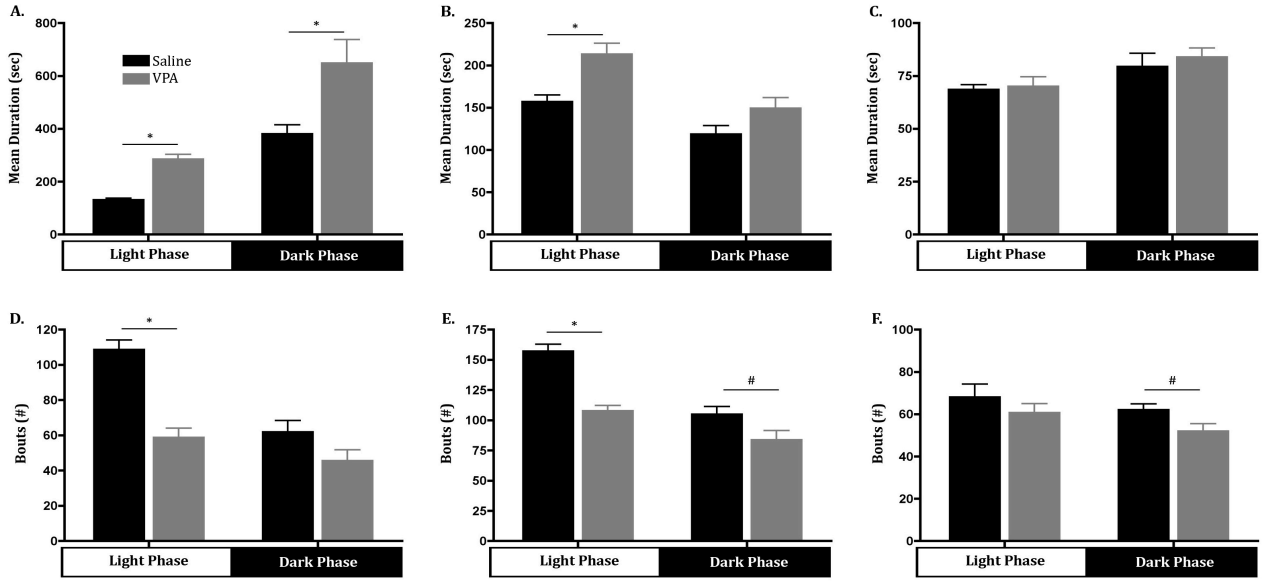

Figure 5. Sleep architecture in VPA and Saline exposed rats. Mean bout duration (A-C) and bout number (D-F) across WAKE (A, D), NREM (B, E) and REM (C, F) in the light and dark phase.

Finally, to assess whether VPA exposure affects the stability of maintaining a wake or sleep state, the number of transition into and out of wake, NREM and REM sleep were quantified across the light and dark phases. In the light phase, the number of transitions to and from wake were significantly reduced by approximately 50% in VPA exposed animals compared to the SAL exposed animals (Table 2 wake to NREM: t(9)=6.460, p=0.0001, NREM to wake: (t(10)=6.509, p=0.0001, and REM to wake: t(10)=3.484, p=0.007]). The transitions during the 12 hour dark phase were not significant differences between control and VPA treated animals.

	Light Phase		Dark Phase	
Transition	Saline	VPA	Saline	VPA
Wake to NREM	**107.6 ± 5.4**	**58.0 ± 5.3**	61.0 ± 6.9	45.0 ± 6.5
NREM to Wake	**88.0 ± 4.6**	**46.0 ± 4.5**	42.6 ± 4.4	32.2 ± 5.1
NREM to REM	68.0 ± 6.2	60.7 ± 4.3	*61.4 ± 3.1*	*51.8 ± 3.6*
REM to Wake	**19.6 ± 2.2**	**11.5 ± 1.1**	18.4 ± 2.5	13.3 ± 1.7
REM to NREM	48.2 ± 5.1	44.3 ± 0.5	43.4 ± 2.3	38.5 ± 4.3

Table 2. Mean number of transitions during average 12 hour light phase and Dark phase (day 1 and 4 averaged). The number of transitions from wake to NREM, NREM to wake, and REM to wake are significantly less in VPA-treated animals compared to controls in the light phase. There were no significant differences in the dark phase.

Task 2: Examine changes in cytokine expression across a developmental time period.

Design: In this task, offspring from the dams treated with either VPA or saline as described above were collected at postnatal day PN 5, PN 20, PN 35, and PN 45. Micropunches of basal forebrain/ preoptic area (BFB/POA sleep active) cortex (arousal) , and PVN (control)were taken. Levels of TNF-alpha, IL-1beta and IL-10 were analyzed via ELISA.

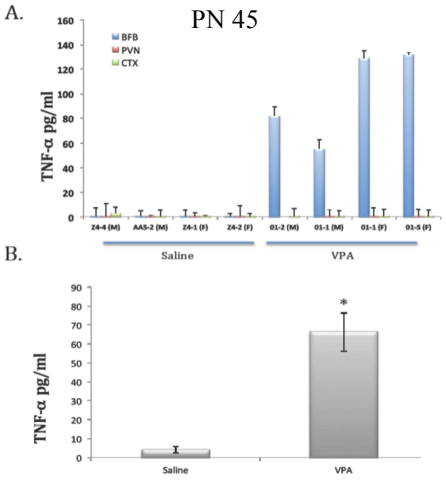

Figure 6: TNF-alpha levels at PN 45 in offspring exposed to VPA or saline in utero. (A) Representative levels in the basal forebrain/POA (BFB/POA), paraventricular nucleus (PVN) and cortex (CTX) of TNF-alpha from individual animals exposed to either saline or VPA. (B) Mean amount in pg/ml of TNF-alpha in the BFB. Data are represented as Mean +SD. *p=0.04, two tailed t-test.

Results: *TNF-α is increased in offspring exposed to VPA in utero at PN45.* Analysis via ELISA plates revealed a significant increase in the amount of TNF-α in the basal forebrain (BFB/POA; Figure 6A, B) at PN 45, but not the cortex or PVN (Figure 6A). On PN 45 there were no significant differences detected in the cortex or PVN (Figure 6A).

The increase in TNF-α in the BFB/POA is significant since this is a site of integration for brainstem arousal-REM circuits. The BFB/POA signals to the cortex and is believed to play a role in sleep-wake transitions.

At earlier time points, no significant differences were observed in TNF-α levels between VPA and saline-exposed groups in the BF/POA, CTX or PVN. On PN 35, levels showed an increase but did not reach statistical significance (Figure 7).

Finally, we did not detect any differences in any region or at any age for IL-10 and IL-1 beta. The pg/ml of each cytokine for all conditions was either not detectable or below the level of the threshold of the assay suggesting that these cytokines have a much lower level of expression tan TNF alpha. A more accurate assessment of these cytokines may require pooled tissue in order to achieve consistent detectable levels.

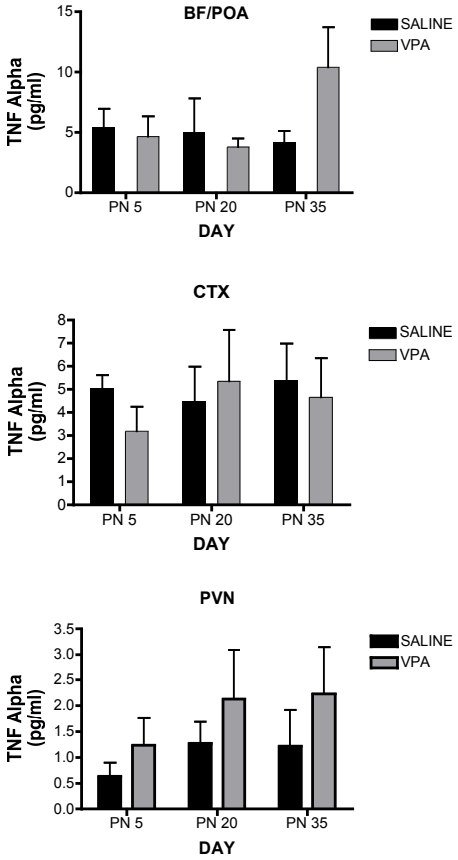

Figure 7: TNF-alpha levels at PN 5, PN 20 and PN 35 in offspring exposed to VPA or saline in utero. There were no significant changes detected in the BFB/POA, CTX or PVN at any age as determoined by a two-tailed t-test, However, in the BFB/POA on PN 35 there was an increase. Data are represented as Mean +SD.

Task 3: Investigate the direct role proinflammatory cytokines in sleep dysregulation in an ASD rodent model.

Design. In order to more directly test whether an increase in proinflammatory cytokines as a result of in utero exposure to VPA underlies the changes in sleep architecture we attempted to block the TNF-α in the basal forebrain. We attempted this blockade by administering blocking antibodies against TNF-α intranasally.

Experimental Paradigm. All surgical manipulations occurred on a single day to avoid multiple doses of anesthesia. Sprague-Dawley rats were fitted with telemetry transmitters on PN 35 as described in Objective 1. Following surgery, the test animals were returned to their home cage and maintained on a 12 hour light/dark cycle. EEG recording were collected on PN 40-45. We found that VPA exposed animals with (n=4) or without blocking antibodies (n=4) did not significantly differ in the amount of time they spent in wake, NREM or REM (two tailed t-test; Table 3) suggesting that either our intranasal administration was not effective or that elevated cytokine levels do not contribute to the noted sleep disruptions. Because we could not measure the effective of the blocking antibodies, a better approach to testing this question would be to surgically cannulate the

TABLE 3: Mins spent in Wake, NREM and REM

	CONTROL (VPA +VEH)		Blocking (VPA +AB)	
	Light Phase	*Dark Phase*	*Light Phase*	*Dark Phase*
WAKE (mins)	258±97.5	466±57.6	288±33.4	493±39.3
NREM (Mins)	375±99.4	236±60.0	386±18.5	186±46.1
REM (Mins)	40±3.4	17.7±5.7	38±7.9	18.7±6.8

animals and microinject the blocking antibodies directly into the target nuclei.

Task 4: Investigate the direct role of specific sleep states on ASD associated behaviors in a rodent model.

Design. In this task we sought to examine the effects of pharmacologically induced REM (with carbachol or vehicle) and NREM (with PGD_2 or vehicle) sleep on ASD associated behaviors (social behavior and locomotor paradigms) VPA or saline exposed animals. To do so, this required forebrain (NREM) brainstem (REM) sleep centers to be canulated in addition to having EEG electrodes placed on the skull. We made numerous attempts to successfully canulate and inject the drugs into PN 35 animals. Unfortunately, in most attempts the animals did not survive the surgeries (possibly because of the age and small body size). Each of the target nuclei is located near respiratory control centers. We found that either during canulation or shortly thereafter during recovery the animals stop breathing suggesting that we are damaging these centers.

One possible way around this technical problem would be first to characterize the sleep patterns in older animals that have been exposed in utero to VPA. If the sleep patterns are similar to the juvenile ones we have discovered, then cannulate and inject the drugs. In fact, for another sleep study (unrelated to this work) we have successfully cannulated adult (PN 95) animals suggesting that this approach is more sound than treating juveniles.

Key Research Accomplishments

- In utero exposure to VPA results in sleep dysregulation in the exposed offspring.
- In utero exposure to VPA results in an increase in the proinflammatory cytokine TNF alpha.
- However, this increase in TNF alpha appears not to be involved in sleep dysregulation
- This VPA model will be a useful tool in further exploring the etiology of sleep disruption in ASD

Reportable Outcomes

D.M. Cusmano, M.A. Castello & J.A. Mong. *In utero* exposure to valproic acid changes sleep patterns in juvenile rats: a potential model for studying sleep disturbances in autism spectrum disorders. Program No. 906.11, 2010 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010.

D.M. Cusmano, M.A. Castello & J.A. Mong. *In utero* exposure to valproic acid changes sleep patterns in juvenile rats: a potential model for studying sleep disturbances in autism spectrum disorders. Program No. P024, 2010 Brain Research Meeting Program. San Diego, CA: Brain Research meeting: The emerging neuroscience of autism spectrum disorders: etiologic insights; treatment opportunities, 2010.

D.M. Cusmano, M.A. Castello, S.S. Viechweg, & J.A. Mong. Sleep patters are changed in a developmental model of autism spectrum disorder. Program No. P3.30, 2010 SBN Program Book. Toronto, ON, CA: Society for Behavioral Neuroendocrinology, 2010.

D.M. Cusmano, M.A. Castello & J.A. Mong. *In utero* exposure to valproic acid changes sleep patterns in

juvenile rats: a potential model for studying sleep disturbances in autism spectrum disorders. Program No. 15, 2010 Program in Neuroscience Retreat Program. Baltimore, MD: Program in Neuroscience 13th Annual Retreat, 2010.

Conclusion

Taken together these data suggest that the VPA treated animals have consolidated periods of wakefulness that may be analogous to insomnia-like behavior. To our knowledge this is the first report of an accepted animals model for ASD exhibiting changes in sleep architecture that mimic that of clinical reports.

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Appendices

1. Society for Neuroscience Abstract
2. Brain Research Meeting Abstract
3. SBN Abstract
4. Program in Neuroscience Abstract

Society for Neuroscience

In utero exposure to valproic acid changes sleep patterns in juvenile rats: a potential model for studying sleep disturbances in autism spectrum disorders.

Danielle M. Cusmano[1,2] Michael A. Castello[2], Jessica A. Mong[2]

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Sleep disruptions are a common clinical feature observed in children with autism spectrum disorders (ASD). These include irregular sleep-wake patterns, delayed sleep latencies, and problems with sleep maintenance. The etiology of these sleep disturbances is unknown and remains relatively unexplored in any animal model of ASD. Prenatal valproic acid (VPA) exposure is a proposed model of ASD. Pups exposed to VPA *in utero* show similar characteristics to children with ASD, including abnormalities in brain morphology and sex-specific behavioral deficits. With this model, we examined the sleep architecture of prenatally, VPA- treated juvenile rats (PN31-34). We used a telemetry system to record electroencephalogram and electromyogram activity of each animal. Two 12-hour light phases (PN 31 and PN 34) were manually scored for each vigilance state and the data were averaged for each animal. VPA-treated animals showed a change in sleep patterning, with more consolidated bouts of wake (60.1% increase in average bout length; $t_{(11)}$=5.783, p=0.0001) and non-REM sleep (21.8% increase; $t_{(11)}$=4.066, p=0.0019) as well as fewer transitions into wake and non-REM sleep. There was a significant decrease in the number of transitions from wake to non-REM sleep (36%; $t_{(11)}$=8.657, p<0.0001), non-REM sleep to wake (30.7%; $t_{(11)}$=6.974, p<0.0001), and REM sleep to wake (52.8%; $t_{(11)}$=5.712, p=0.0001). Total sleep time and delta power, however, were similar in VPA-treated and controls animals. While these disruptions did not alter the total amount of acquired sleep in the animal's sleep phase, the alterations in patterning may disrupt sleep-dependent synaptic downscaling possibly leading to abnormal dendritic arborization and connectivity. By better understanding the consequences of disrupted sleep patterning, potential sleep therapies may be used to help alleviate some of the symptomology associated with ASD.

Brain Research Meeting

In utero exposure to valproic acid changes sleep patterns in juvenile rats: a potential model for studying sleep disturbances in autism spectrum disorders.

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Sleep disruptions are a common clinical feature observed in children with autism spectrum disorders (ASD). These include irregular sleep-wake patterns, delayed sleep latencies, and problems with sleep maintenance. The etiology of these sleep disturbances is unknown and remains relatively unexplored in any animal model of ASD. Prenatal valproic acid (VPA) exposure is a proposed model of ASD. Pups exposed to VPA *in utero* show similar characteristics to children with ASD, including abnormalities in brain morphology and sex-specific behavioral deficits. With this model, we examined the sleep architecture of prenatally, VPA- treated juvenile rats (PN31-34). We used a telemetry system to record electroencephalogram and electromyogram activity of each animal. Two 12-hour light phases (PN 31 and PN 34) were manually scored for each vigilance state and the data were averaged for each animal. VPA-treated animals showed a change in sleep patterning, with more consolidated bouts of wake (60.1% increase in average bout length; $t_{(11)}$=5.783, p=0.0001) and non-REM sleep (21.8% increase; $t_{(11)}$=4.066, p=0.0019) as well as fewer transitions into wake and non-REM sleep. There was a significant decrease in the number of transitions from wake to non-REM sleep (36%; $t_{(11)}$=8.657, p<0.0001), non-REM sleep to wake (30.7%; $t_{(11)}$=6.974, p<0.0001), and REM sleep to wake (52.8%; $t_{(11)}$=5.712, p=0.0001). Total sleep time and delta power, however, were similar in VPA-treated and controls animals. While these disruptions did not alter the total amount of acquired sleep in the animal's sleep phase, the alterations in patterning may disrupt sleep-dependent synaptic downscaling possibly leading to abnormal dendritic arborization and connectivity. By better understanding the consequences of disrupted sleep patterning, potential sleep therapies may be used to help alleviate some of the symptomology associated with ASD.

Society for Behavioral Neuroendocrinology

SLEEP PATTERS ARE CHANGED IN A DEVELOPMENTAL MODEL OF AUTISM SPECTRUM DISORDER

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Autism spectrum disorders (ASD) are an array of developmental disorders, primarily disrupting social interactions, communication skills, and patterns of behavior. ASD is four times more prevalent in boys (4:1) than girls. Clinical observations have suggested that there is a higher prevalence of sleep disturbances in children with ASD compared to normally developing children, including difficulties initiating sleep and waking periodically throughout the night and early morning. The etiology of these sleep disturbances is unknown and remains relatively unexplored in animal models of ASD. Prenatal valproic acid (VPA) exposure is a well-accepted model of ASD. Pups exposed to VPA *in utero* show similar characteristics to children with ASD, including abnormalities in brain architecture and sex-specific behavioral deficits. With this model, we examined the sleep architecture of VPA-treated juvenile rats (PN31-38). VPA-treated rats showed a significant increase in the total time spent in wake at the expense of slow wave sleep compared to their age-matched controls. While the current data set did not allow for analysis of sex differences in VPA animals, preliminary analysis suggest a sex difference in total time spent in wake in controls. These data suggest that VPA treated animals may have disrupted sleep patterns and that a sex difference may exist in juvenile sleep. Future experiments will explore the role of hormonal and developmental factors on sleep patterning in both VPA and normally developing rats.

Program in Neuroscience Abstract

In utero exposure to valproic acid changes sleep patterns in juvenile rats: a potential model for studying sleep disturbances in autism spectrum disorders.

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Sleep disruptions are a common clinical feature observed in children with autism spectrum disorders (ASD). These include irregular sleep-wake patterns, delayed sleep latencies, and problems with sleep maintenance. The etiology of these sleep disturbances is unknown and remains relatively unexplored in any animal model of ASD. Prenatal valproic acid (VPA) exposure is a proposed model of ASD. Pups exposed to VPA *in utero* show similar characteristics to children with ASD, including abnormalities in brain morphology and sex-specific behavioral deficits. With this model, we examined the sleep architecture of prenatally, VPA- treated juvenile rats (PN31-34). We used a telemetry system to record electroencephalogram and electromyogram activity of each animal. Two 12-hour light phases (PN 31 and PN 34) were manually scored for each vigilance state and the data were averaged for each animal. VPA-treated animals showed a change in sleep patterning, with more consolidated bouts of wake (60.1% increase in average bout length; $t_{(11)}$=5.783, p=0.0001) and non-REM sleep (21.8% increase; $t_{(11)}$=4.066, p=0.0019) as well as fewer transitions into wake and non-REM sleep. There was a significant decrease in the number of transitions from wake to non-REM sleep (36%; $t_{(11)}$=8.657, p<0.0001), non-REM sleep to wake (30.7%; $t_{(11)}$=6.974, p<0.0001), and REM sleep to wake (52.8%; $t_{(11)}$=5.712, p=0.0001). Total sleep time and delta power, however, were similar in VPA-treated and controls animals. While these disruptions did not alter the total amount of acquired sleep in the animal's sleep phase, the alterations in patterning may disrupt sleep-dependent synaptic downscaling possibly leading to abnormal dendritic arborization and connectivity. By better understanding the consequences of disrupted sleep patterning, potential sleep therapies may be used to help alleviate some of the symptomology associated with ASD.